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In silico bioprospection analysis for identification of herbal compound targeting *Clostridium difficile*

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Clostridium difficile infection (CDI), the occurrence has been increasing in the community as well as hospital-care facilities, resulting in higher morbidity and mortality rate. *C. difficile*, anaerobic pathogen greatly associated with antibiotic resistance and majorly linked to the irrational antibiotic use, which accelerate the alarming situation causing endemic as well epidemic globally. It is the budding menace and one of the major sources of nosocomial infection, i.e., *hospital-acquired infection*. The prevailing risk to public health by the antibiotics and their resistance majorly has driven the urge for utilizing the traditional herbal medicine into a sophisticated approach as a Modern/Ayurvedic Medicinal System (AMS). The current study aims to find out the promising herbals to combat the threat caused by *C. difficile* by applying herbal informatics as a holistic approach. Total 44 plants were elucidated against the virulence factors of the bacterium using the systematic bioprospection approach, out of which 5 plants were optimized that may be futher validated at the preclinical level.

Keywords*:* Ayurvedic Medicinal System (AMS), *Clostridium difficile*, Ethnopharmacology, Herbal bioprospection, Multi-drug resistance, Nosocomial

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Multi-drug resistant *C. difficile*, is a gram-positive, spore-forming anaerobic bacteria, was first identified in the 1930s and recognized as a human illness and mortality cause in 1978[1]. Clinically, *C. difficile* may present in latent form, but can cause severe life-threatening diseases like colitis, diarrhea, toxic megacolon, fever, etc[2]. The statistical data of hospital-acquired infections from last two decades around the world have been documented with a very high prevalence rate of illness associated with *C. difficile*[3]. The prevalence rate of infection caused by *C. difficile* in India is 1.67% which is similar to the USA, i.e., 1.6%[4]. As per 2013 Centre for Disease Control (CDC) report, *C. difficile* categorized under the "urgent threat" along with Carbapenem-resistant Enterobacteriaceae (CRE) and Drug-resistant *Neisseria gonorrhoeae*[5].

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Irrational use of antibiotics is one of the major reasons that make the condition more dreadful in treating *C. difficile* infection (CDI)[6]. Metronidazole and vancomycin are the principal drugs used to treat CDI. Discontinued use of antimicrobial drugs accelerates the emergence of drug-resistant strains, reached a critical level globally[7]. Though there are many antimicrobial agents available and new antibiotics are under research but still, it is one of the second cause of death worldwide due to a resistance phenomenon[8].

Due to a frequent relapse rate of infection and multiple drug resistance[9], a new approach using traditional regimes have been practiced such as AMS[10]. Herbal use in the management has acquisition attention worldwide by making the use of herbals[11]. Almost 420,000 medicinal plant species exist on the planet, that are foreseen by various researchers and scientists across the world, but it has been predicted that only 10% of plants, been explored and investigated upon for their medicinal usage[12].

According to the World Health Organization (WHO) reports, the remedies that are fortified from a natural source, cater to only 3.5 to 4 billion people globally and is used for primary healthcare[12]. Medicine obtained from herbal works on the holistic approach to combat the CDI. The current study emphasizes the rationale to implement the herbal informatics approach to bioprospecting, determine, scrutinize and validate the AMS, based on the ethnopharmacological properties, as appeared in *vedic* literature. The leads/drug-like herbals that are identified by herbal informatics model, could be valuable at *in vitro* and *in vivo* study for discovery of the curative herbal drug.

Methodology

Classic literature surge model

Bioactivity parameters were selected based on virulence of the pathogen by classical bioprospection approach and the classic literature surge that involves the sympathetic of the various characteristics of infection caused by *C. diffcile*[13].

Evaluation of relevance factor using 'keywords hits' scoring matrix approach

Academic Search Engine Optimization (ASEO)[14] is accomplished by scheming percentage relevance of the bioactivity parameters using PubMed (http://www.ncbi.nlm.nih.gov/pubmed) as a search engine tool. ASEO is a work on the grading of virulence factors on the top position that is most expected to be therapeutic targets followed by linking with the observation which was based on the analysis of the first 20 hits, relevance factor/ net weightage of each virulence factor was evaluated using the following formula.

$$Percentage\ relevance = \frac{Relevant\ Hits \times 100}{20}$$

Bioprospection approach for identification of herbal compounds

The classical bioprospection approach accounts for investigation of the following variables based on a literature review to devise a logical conclusion, resultant in the selection of plants. It includes:

a) Ethnopharmacological importance

b) Relevance of Herb in traditional medicine

c) Availability factor or cultural acceptability in localized regions

d) Any Vedic literature supporting its use

e) Investigations/ prior experience on the potential of the herb

f) Indirect indications, if any, etc[15].

Binary coefficients matrix to assess the presence/absence of a Bioactivity parameter (BAP)

Binary coefficients matrix works on the principle of the 0-1 score of a BAP. Based on this step, herbals having >03 BAP, selected in PubMed search engine (n= first 20 hits) against 'Bioactivity Parameter + Selected Plant' random search model, were selected. This is the steps forward to support the holistic approach and scrutinize the lead for next-level analysis, in line with the validation of the current study.

Weightage matrix-based scrutiny

Assessment of overall weightage of plants (scores ≥ 3 in the previous step) was validated by multiplying their binary score with the weightage (as obtained in above step). This step further augments the 'uncertainty factor' that is essential for the statistically significant outcome and also identifies the potential drug-like herbals to cross-validate at next level Fuzzy set optimization.

Fuzzy set optimization for the decision matrix

The mathematical relationship as mentioned below was used to ascertain relative relevance within an identified set of herbals.

$$\mu S = Score – MinS/MaxS – MinS$$

where µS represents the desired values of the selected NPPs of the Fuzzy set.

MinS and MaxS are the minimum and the maximum scores, respectively in Fuzzy sets. The estimated µS were converted into a leveled score by using a scaled magnitude as optimization of identifying a potential set of NPPs.

Results

Bioactivity parameters analysis using the classical approach

Seven testing parameters were selected including inhibition of (i) Adhesion (ii) Enterotoxin (iii) Cytotoxin (iv) Quorum sensing (v) Binary toxin (vi) Capsular polysaccharide and (vii) Symptomatic relief provision.

Keywords hit scoring matrix approach to evaluate the relevance factor

This matrix analysis provides weightage score to numerous parameters which were recognized for scrutinizing herbal compounds concerning their antibacterial efficacy, as shown by example in Table 1. According to the percentage (%) relevance approach, weightage was given to each parameter. Highest relative percentage relevance was obtained for Quorum Sensing Inhibitor (QSI) (i.e., 7), Capsular polysaccharide inhibition (CPI) (i.e., 5.185), followed by other parameters like Symptomatic Relief (SR) (4.469), Binary toxin Inhibition (BTI) & Enterotoxin Inhibitors (tcdAI) (4.916), Adhesion Inhibition (AI) (3.871), Cytotoxin Inhibition(tcdBI) (2.0830).

Binary matrix-based study

A dataset of 44 herbals was used in this approach that contains ethnopharmacological activity for targeting the virulence factor of CDI as exemplified in Table 2. Out of 44 herbals, 19 most efficacious herbals unveiled a binary score of ≥3.

Simple additive weighting matrix

At this step, the combined weightage scores of 05 herbals found to have significant ($p<0.05$) potential based on their median value score, *i.e.*, 9.829, e.g., *Curcuma longa, Zingiber officinalis, Camellia sinensis*, *Vaccinium macrocarpon* and *Piper longum*. This herbal indicates therapeutic activity against the multi-drug resistant pathogens.

Decision matrix-based Optimization

Decision matrix and its analysis simplified that higher percentage relevance score directly indicates the therapeutic potency against CDI. However, the 05 plants were found to have higher optimization score as mentioned in Table 3. However, *Rosmarinus offcinalis* showed the highest fuzzy score (µS) equal to 01, whereas *Glycyrrhiza glabra* exhibited the lowest µS score was 0.72. The fuzzy analysis was further utilized to obtained the optimized scores (range 0-1) for the selected plants to provide a collectively acceptable optimized score which converts all the weightage score within 0-1 range and could be utilized as the decision-maker for herbal compounds at *in vitro* and *in vivo* study for drug development or supplement process.

Discussion

Antibiotics invention is considered as one of the biggest achievements of science as this gave a ray of hope to coup over the range of life-threatening pathogens[16]. The history is witnessed for several such discoveries and victories of such antimicrobial agents from different sources. But the emergence of drug resistance makes the condition more outrageous and warrants researcher to find out some novel modalities that can challenge these persistent life-threatening

Table 1 — Rationale for selection of the bioactivity parameters (BAP) for bioprospection study

S.No	BAP	Rationale for selection
1	Adhesion Inhibition (AI)	• a.The first step in the sequence of events leading to colonization. • b.*Zingiber officinalis* are known to enhance cell wall permeability.
2	Enterotoxin Inhibition (tcdA-I)	• a.*Clostridium difficile* possesses potent toxin which act by modifying GTPase proteins by glycosylation leading changes in cellular activity. • b. *Zingiber officinalis, Piper longum,* Cranberry are known to act as antitoxin which inhibit the glycosylation.
3	Cytotoxin Inhibition (tcdB-I)	• a. Cause disruption of actin cytoskeleton and produce inflammatory changes in host. • b. *Camellia species, Acacia fernisiana* against tcd B toxin of *Clostridium difficile* could suppress the inflammatory changes by nutilizing the toxin release.
4	Quorum Sensing Inhibition (QSI)	• a. *Clostridium difficile* used processed oligopeptides to communicate via auto-inducers. • b. Plants like *Zingiber officinalis* and *Citrus sinensis* inhibits Quorum sensing mechanism and preventing replication.
5	Binary Toxin Inhibition (BTI)	• a. Highly virulent group of strain caused epidemic and outbreaks in Europe. • b. Herbals not showed for inhibiting the toxin of CDI .
6	Capsular Polysaccharides Inhibition (CPI)	• a. *Clostridium difficile*, pathogenic bacteria produce capsular polysaccharides virulence factors. • b. Herbals like *Camellia sinensis* showed inhibitory activity on capsular polysaccharide.
7	Symptomatic relief (SR)	• a. *Clostridium difficile* associated bloating, diarrhea, *pseudomembranous colitis* are treated using these phytochemical based modalities like *Artemisia maritima,* etc

Table 2 — Selected herbal plants showing probable ethanopharmacological utility

S.No	Botanical plant	Common name	Predominant phyto constituents	Parts utilized	Availability	Relevance of herb in traditional medicine	Current indications
1	*Artemisia ludovician Nuff.*	*Silver wormwood, Sulasi*	Methyl homo anisic acid, cineol and linalool, Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)	Roots, leaves	Found throughout the Philippines and North America.	Relieves pain during menstrual cycle.	Antifertility, anticancer, antidiabetic, antifungal, antimicrobial, galactagogue, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic actions.
2	*Artemisia maritima L.*	Seawormwood, oldwoman	Alkaloid santonin		Western Indian Himalaya	Used against liver damage	Antibacterial, antifungal
3	*Camellia sinesis (L.) Kuntze*	Green Tea	Caffeine, Theobromine, Theophylline, Purine derivatives like xanthine etc.	Leaves	Originally from the triangle of countries of South China, Assam (northeastern India) and Cambodia.	Promote blood circulation, promote excretion of alcohol and other harmful substances, invigorate the skin, promote digestion, combat tiredness and depression, among many others.	Anti oxidant, anti diabetic, hypolipidemic, antibacterial, hepatoprotective.
4	*Cinnamomum verum*	Cinnamon, Tamalpatram	eugenol,linalol	Bark	China, India , Vietnam	Flavouring agent and used as alcoholic beverage	Anti-dysentry, antibacterial
5	*Citrus sinensis (L.) Osbeck*	Orange	Artemisinin, arteether, artemether etc.	Dried outer covering	Grown in China, Japan, Germany, Korea	Aqueous preparations of the dried herb were applied against fever, malaria, skin diseases, jaundice and haemorrhoids.	Antiangiogenesis effects, Antimalarial effects, Antioxidant, Anticancer, Antimicrobial.
6	*Corymbia citridora (Hook.)*	Lemon Eucalyptus.	Citronella, citronellalisovalererie	Leaves, bark, seed	North eastern Australia, Asia	Used treat diabetes	Antihelmantic, anti-inflammatory
7	*Curcuma longa L.*	Turmeric	Curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone, and zingiberone.	Rhizome, leaves	Native to India. Widely distributed in the Philippines.	Decoction of rhizome, as tea, used for fevers, dysentery, abdominal pain, flatulence, abdominal spasm, arthritis.	Antioxidant, antiinflammatory, cholesterol-lowering, antibacterial, antifungal, antiviral, immunomodulatory, hepatoprotective, and anticarcinogenic activity.
8	*Glycyrrhiza glabra L.*	Liquorice	Glycrhirrhizin Glycyyrhizic acid	Roots,leaves and rhizome	Cultivated everywhere,native of southwest Asia		Antibacterial, hepatotoxic
9	*Humulus lupulus L.*	Common hop.	Polyketides, alkylated flavanones, anthraquinones		Central Europe, USA and India	Traditionally used to treat insomnia,relieve the pain of bladder infection	Anti-inflammatory Activity, Anti-anxiety,muscle relaxant

(*contd.*)

Table 2 — Selected herbal plants showing probable ethanopharmacological utility (*contd.*)

S.No	Botanical plant	Common name	Predominant phyto constituents	Parts utilized	Availability	Relevance of herb in traditional medicine	Current indications
11	*Momordica charantia L.*	Bitter gourd	dihydrophaseic acid, magnesium, calcium	Fruit, leaves, stem	Widely cultivated in the world.	Widely grown and cooked for various diseases such as colitis, dysentery, viral infections, cough, flux.	Anti-bacterial, anti-oxidative, anti-fugal
12	*Myristica fragrans Houff.*	Nutmeg		Seed	Indigenous to banda islands in moluccas	Vegetables puddings and stews	Abortifacient, intoxicant
13	*Nymphaea tetragona (Aif.)*	White lotus,water lily	tannic acid, gallic acid and few non toxic alkaloids	Roots	Native from the Indian subcontinent to Australia.	Traditionally it is used in the treatment of of bronchial congestion	Antibacterial, anti diarrhea, posses astringent, demulcent and antiscrofulousactivityl
14	*Papaver somniferous L.*	Opium	Morphine,codeine, Noscapine	Capsules	Asia,Afganistan,	Sedative, euphoric, recreational purposes	
15	*Piper longum L.*	Black mustard	Phytoalexins (sinalexin, sinalbins A and B), sterols and steryl esters (primarily sitosterol and campesterol), and flavonoids (eg, apigenin, chalcone).	Dried seeds	Native to the southern Mediterranean region of Europe and possibly South Asia	Food flavoring, for forage, as an emetic, and diuretic, as well as a topical treatment for inflammatory conditions such as arthritis and rheumatism.	Antibacterial, Hypergylcemic and cardiovascular effects
16	*Vacccinium macrocarpon L.*	Cranberry	Polyphenol, tannins, flavanols	Stem bark	Native to primarily North America, primarily Canada and central United States.	Treatment of bladder and kidney	Antiamebic, antianemic, antibacterial, anticonvulsant, antidiabetic, antidiarrheal, antihelminthic, anti-inflammatory, antimalarial, antinociceptive, antiviral, and hypoglycemic effects.
17	*Vachellia fernesian (L.) wight & Arn.*	Sweet acacia, needlebush	Ellagicacid, methylgallate,gallic acd, naringin-7-diglucoside	Bark, flower and seed	Australia,southernasia	Muscle relaxant, cardiacdepressant, antihelmintic, antidysentric	Muscle relaxant, cardiacdepressant,antihelmintic,antidysentric
18	*Zataria multiflora Boiss.*	Satar	thymol,carvaenol	Leave	Southern-west asia	Whooping cough , sore throat	Anti-nociceptive Antispasmodic
19	*Zingiber officinale Rosc.*	*Ginger ,aada*	Zingerone, Gingerols	Rizome	Grows in india,China, Nepal, etc.	Effective for treating nausea caused by morning sickiness,sea sickness	Antibacterial, antihelminthic, antimycotic, antiviral, antispasmodic, diaphoretic, expectorant, fibrinolytic, hypotensive, promoting leucocytosis, lipid lowering and platelet aggregation inhibition.

Table 3 — Fuzzy set membership analysis for herbal on the basis of weightage matrix scores

S.No	Herbal plants	Code	μS	Optimized score
1	*Zingiberofficinalis*	*Zf*	1	+++++ (5)
2	*Curcuma longa*	*Cl*	0.9673817	+++++ (5)
3	*Camellia sinensis*	*Cs*	0.9673088	+++++ (5)
4	*Piper longum*	*Pl*	0.64	++++ (4)
5	*Vaccinium macrocarpon*	*Vm*	0.64	++++ (4)

microbes[17]. While on the other hand, the ethnopharmacological importance of several medicinal plants and its proper implementation as in medicine is still a matter of deliberation. This study is a presentation of *in silico* model which is done by utilizing *in silico* bioinformatic method to get auspicious results. This process includes the BAP by literature study, their indexing status and collection of medicinal herbals to prepare a large database. Based on the presence or absence of a particular virulence factors/BAP identification of the potential plants, scoring was given by calculating the decision matrix of their final weightage[18].

The herbal compound was identified utilizing the binary matrix approach based on all or none principle, thus eliminating the herbal compounds having score < 03 median cut-off value. Subsequently, 19 plants out of 44 plants were analyzed to cross-validate the binary scoring of plants for enduring the weightage matrix and the fuzzy set optimization, which provided an expansively satisfactory score for further validation of scrutinized lead at their molecular level.[19].

This bioprospection/herbal informatic analysis provides 05 plants with a therapeutic potential against CDI, *i.e.*, *Camellia sinensis*, *Zingiber officinalis*, *Curcuma longa*, *Piper longum* and *Vaccinium macrocarpon*, which would be a further study for reconnoitering its holistic potential at *in vitro* and *in vivo* level study.

Conclusion

Bioprospection study has revealed 05 Natural Plant Products (NPPs), *i.e.*, *Camellia sinensis*, *Zingiber officinalis*, *Curcuma longa*, *Piper longum*, *Vaccinium macrocarpon*, with significant therapeutic activity against *C. defficile*. These herbals could be further used to validate their curative potential at *in vitro* and *in vivo level*.

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Conflict of interest

None

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